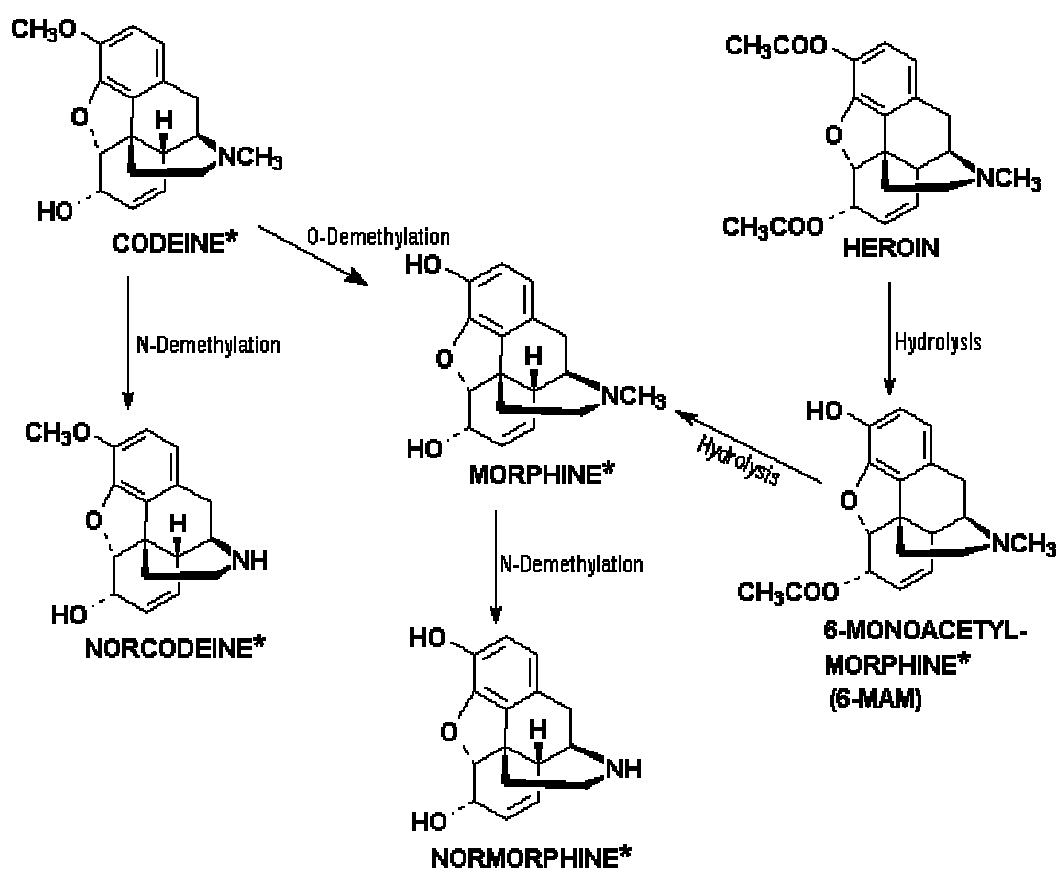


Extraction and Determination of 6-Monoacetylmorphine by LCMS

This procedure describes the extraction and determination of the heroin metabolite 6-monoacetylmorphine in urine by SPE and Liquid Chromatography-Mass Spectrometry (LCMS) with electrospray ionisation using full scan ms/ms detection in the positive ion mode.

The most important indicator of heroin use is 6-monoacetylmorphine. Heroin (diacetylmorphine) is rapidly metabolised in vivo to form 6-monoacetylmorphine which is then further metabolised to morphine. 6-monoacetylmorphine is normally only detected within 12-24hr of heroin use.

Fig 1 Metabolism of Heroin and Codeine



* Glucuronide & sulphate conjugates

The extraction procedure

6-monoacetylmorphine is acid labile and therefore acid hydrolysis cannot be used in the analysis as is usual in the total opiate method.

Deuterated internal standard is added to the specimen which is then passed through SPE cartridges and extracted using an otherwise general opiate extraction procedure. The resultant extract is dried and re-dissolved in a mobile phase (0.1% formic acid/acetonitrile) in a ratio of 70:30.

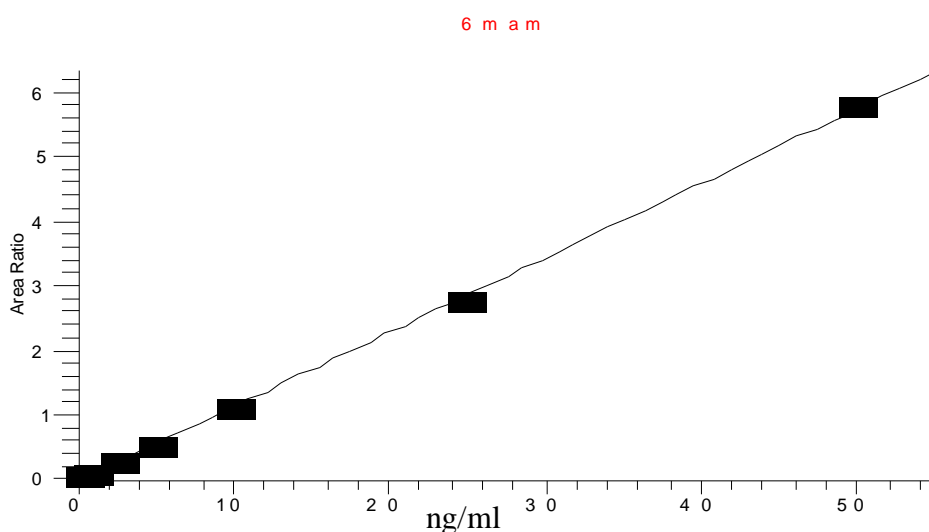
Analysis on LCMS

The extract is then injected into a ThermoFinnigan LCQ Deca XP LCMS system containing a Phenomenex Synergy Hydro RP 50mm, x 2.1mm id 4µm particle size column or equivalent. The LCMS is calibrated daily using 6-monoacetylmorphine standards and quantified by linear regression of the relative areas of standard/internal standard in the specimen against the calibration curve. With a limit of detection of 0.5ng/ml the current cut off is 1ng/ml.

Analysis is carried out using electrospray ionisation with full scan ms/ms. The secondary ms is affected by Collisionally Induced Dissociation by introducing He₂ into the ion trap after the isolation of the M+H ions of interest and application of a resonant waveform voltage to induce dissociation.

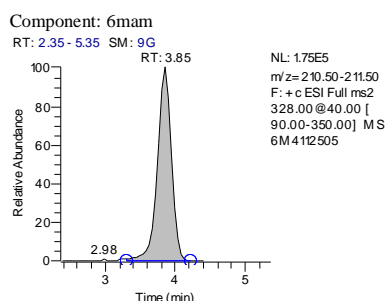
Further research is being carried out in order to detect other heroin metabolites.

Fig 2 Calibration curve for 6-monoacetylmorphine



Equation: $Y = -0.0341153 + 0.115468 * X$ $R^2 = 0.9995$

IT: Method
ERT: 4.00
SW: 90.0
VW: 3.00
RTR: no
ID: Nearest
MPH: 1.0
SM: 9
PDA: Genesis
VD: yes
EPW: 0.0
CP: no



ISTD: d36mam
IT: Method
ERT: 3.90
SW: 90.0
VW: 3.00
RTR: yes
ID: Highest
MPH: 3.0
SM: 9
PDA: Genesis
VD: yes
EPW: 0.0
CP: yes
PH: 1.0
TF: 5.0

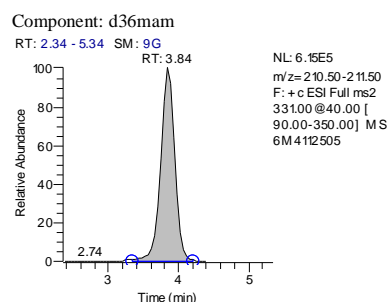


Fig 3 TIC and Extracted Ion Chromatograms of extracted 1ng/ml 6-monoacetylmorphine standard and their corresponding mass spectra

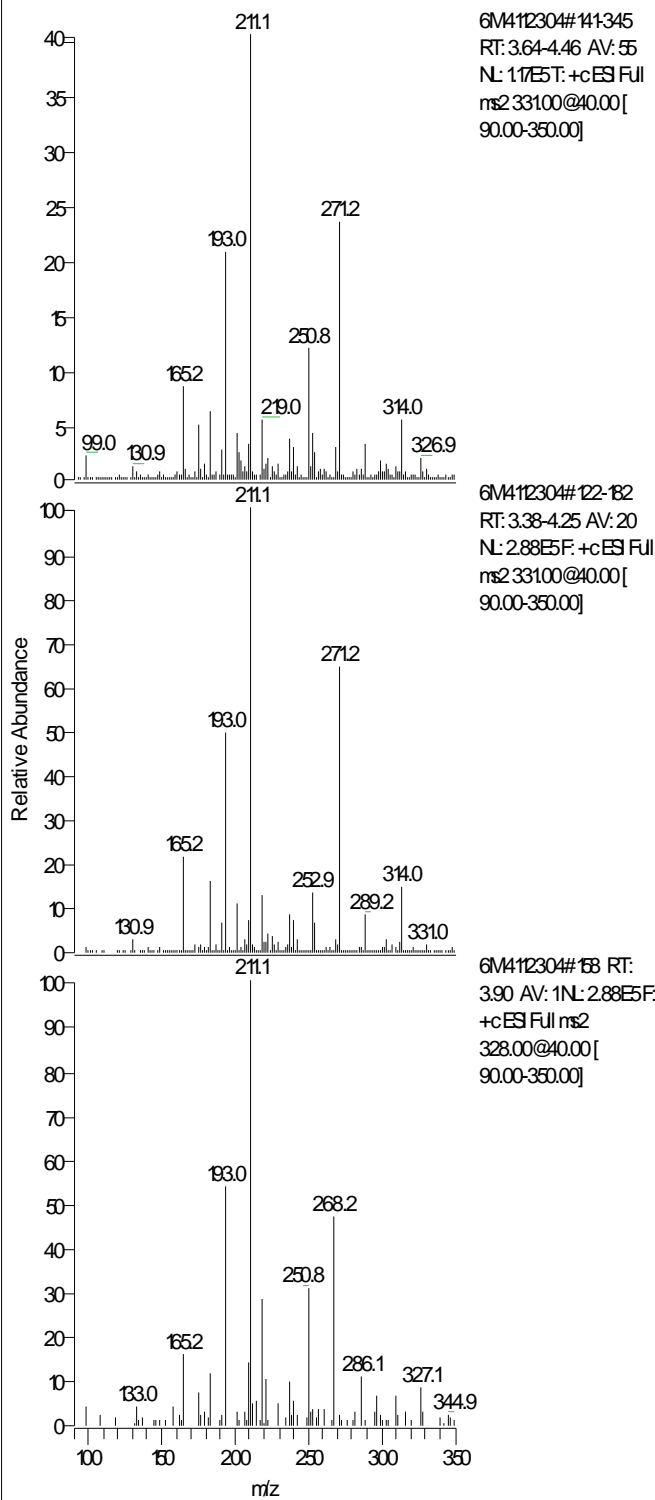
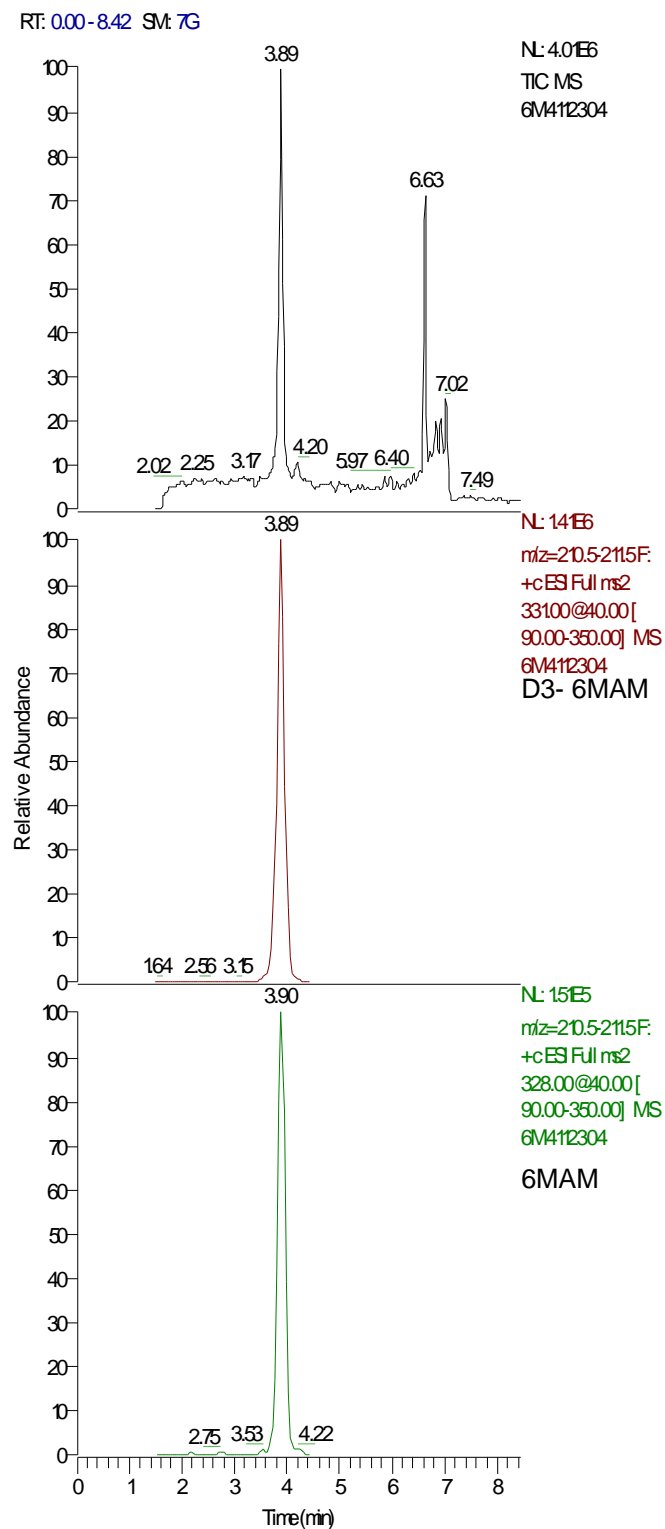


Fig 4 TIC and Extracted Ion Chromatograms of extracted Biorad commercial QC and their corresponding mass spectra

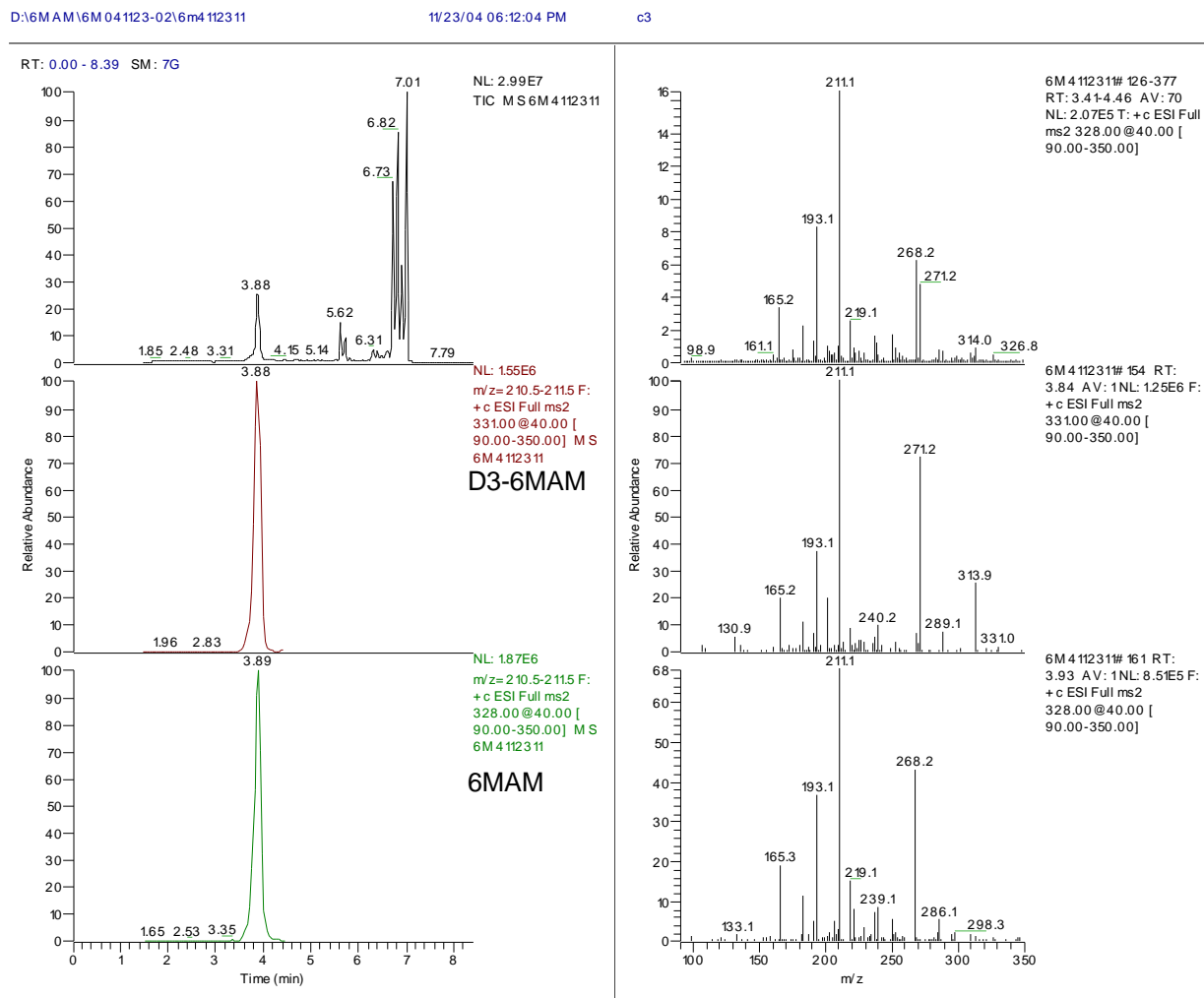


Fig 5 Full scan spectra of 6-Monoacetylmorphine

